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Case report

Fatal and non-fatal cases of lime sulfide exposure and pathogenetic mechanisms underlying pancreatic injury: Case reports with an animal experiment

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ABSTRACT

Lime sulfide poisoning by the oral route is rarely encountered in the practice of forensic science, whereas hydrogen sulfide poisoning is seen frequently. We report here two cases of fatal lime sulfide poisoning with several related cases and in addition induced histological damage with acute inflammation in animal models under at similar concentrations. We also evaluated sulfide and thiosulfate concentrations and speculated as to the cause of pancreatic damage in these cases.

1. Introduction

Sulfide poisoning has hitherto been considered to occur mostly in industrial and various other situations associated with inhalation of hydrogen sulfide,[1–6] but recently a marked increase has also been noted in the number of suicide cases associated with oral ingestion of sulfide-containing household products, notably detergents and bathing items, which are widely available in Japan.[7] In contrast, cases surviving after inhalation of spontaneously generated hydrogen sulfide or cases dying due to direct oral intake of concentrated lime sulfides have been considered to be relatively rare. In this study by focusing on the autopsy findings and measurements of the concentration of sulfides in blood and urine, we were able to elucidate the differences in the mechanisms of death between such cases, and in particular to highlight the significance of direct pancreatic injury resulting in acute pancreatitis as a contributor to the cause of death.

2. Case profiles

2.1. Case 1

A 72-year-old man was found dead in an orchard. He was lying prone completely dressed with a half-full bottle of lime sulfide, an insecticide for gardening containing calcium polysulfides on his right side after ingesting 200 mL of it (Fig. 1A). Police investigation identified the man by his fingerprints. He had been diagnosed with mild dementia and gone missing from a neighboring prefecture. To investigate the cause of death, a medicolegal autopsy was performed 22 h after death.

2.2. Case 2

A 60-year-old woman was found dead in her house after quarreling with her husband. A note was found with a half-full bottle of lime sulfide beside her. A medicolegal autopsy was performed 19 h after death. The autopsies of both Case 1 and 2 were performed at Kanazawa University.

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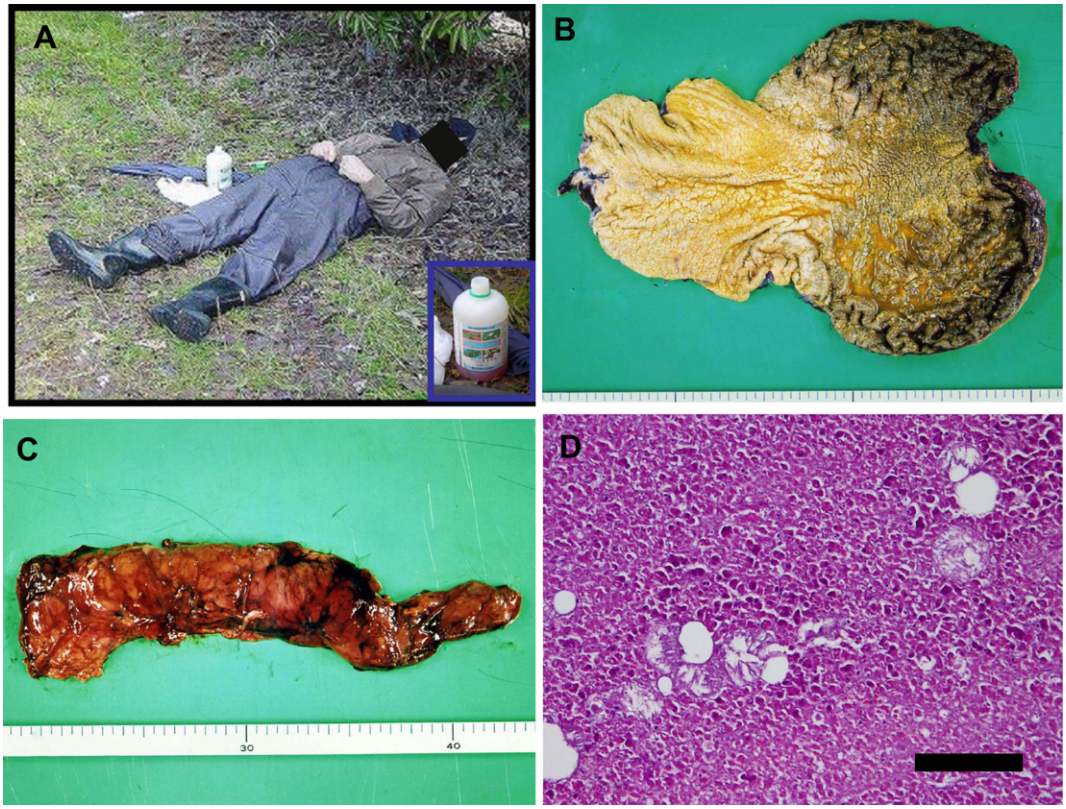

Fig. 1. Pictures of Case 1. A. Scene of Case 1. The corpus with a bottle of lime-sulfur (inset) within his reach. B. Gross appearance of stomach. C. Gross appearance of pancreas. D. Microscopic picture of pancreas shows total necrosis with acute inflammation. Hematoxylin and eosin stain (H&E). Bar = 200 μm.

2.3. Case 3 (committed suicide by inhaling hydrogen sulfide gas)

A 44-year-old man committed suicide by inhalation of spontaneously generated hydrogen sulfide gas. He died soon after informing police by himself. Inspection with sampling of blood and urine was done 5 h after his phone call to the police.

2.4. Case 4 and 5 (survivors of accidents associated with hydrogen sulfide gas inhalation)

Accidental cases at a site for industrial disposal of waste occurred involving 2 persons at the same time. Both were admitted to hospital. Case 4, a man aged 31 years lost consciousness for two days after the incident, whereas Case 5, a man aged 57 years fainted only transiently. The symptoms in Case 5 were mitigated within one day of the accident.

3. Autopsy findings and laboratory data in two of the cases

3.1. Case 1

Macroscopically, the post-mortem lividity was characterized by a combination of greenish-gray and red color. A sulfurous smell was noted when the abdominal cavity was opened. A dark brown fluid permeating around a pale stomach was observed in the abdominal cavity. The esophageal mucosa was partially detached from the submucosal layer, and the stomach showed a firm, leather-like consistency (Fig. 1B).

No alcohol, narcotic drugs, or pharmaceuticals were detected by general toxicological analysis; therefore, lime sulfide poisoning was suspected.

3.2. Case 2

Macroscopically, the color of lividity was greenish-gray and red, and powder-like white residues were found in the mouth. A sulfurous smell was noted when the abdominal cavity was opened. A malodorous dark brown fluid around the perforated stomach and at the ventral aspect of the pancreas was observed in the cavity. The esophago-gastric mucosa was erosive and changed to be firm like leather. The underlying cause of pancreatic injury was direct spread of corrosive lime sulfide through a gastric perforation.

In both cases, every organ, especially the lung and liver, showed marked congestion histopathologically. The esophageal mucosa except for the basal layer was desquamated due to necrosis. In the stomach, there were severe hemorrhage in the entire wall and mucosal necrosis with marked deposition of numerous calcium granules, which appeared as small round particles when stained with hematoxylin. In the pancreas, neutrophil infiltration with widespread necrotic areas was seen. (Fig. 1C, D).

In one of the autopsy cases, blood and urine sulfide levels were not measured, but by spectrophotometry of blood, the peak of absorbance was found at 620 nm, thus showing sulfhemoglobin formation in the blood and establishing sulfide as the source of the poisoning. In 4 of the present 5 cases, sulfide (μmol/mL) and thiosulfate (μmol/mL), which serve as indices of sulfide poisoning, were measured by gas chromatography/mass spectrometry with pentafluorobenzyl bromide derivatization.[8–10] Moreover, since in each of these cases samples were obtained and autopsies performed within one day of the exposure to hydrogen sulfide, generation of sulfide due to putrefaction is considered unlikely.[11,12] In cases committing suicide by hydrogen sulfide gas inhalation, mean values lying between those of surviving cases and cases ingesting lime sulfides were noted (Table 1).

Table 1
Values of sulfide and thiosulfate in the blood and urine.

		Sample	Sulfide (μ mol/mL)	Thiosulfate (μ mol/mL)
Lime-sulfur intake	Case 1	Blood	0.696	0.347
		Urine	0.007	0.067
H_2S suicide	Case 3	Blood	0.269	0.116
		Urine	NA	ca. 0.02
H_2S accident	Case 4	Blood	0.015	0.072
		Urine	ND	2.05
	Case 5	Blood	0.014	0.024
		Urine	ND	0.270

NA, not analyzed; ND, not detected.

As a laboratory parameter of pancreatic injury serum amylase values (normal <166 IU/L) were measured in the surviving cases. Cases 4 and 5 were discharged from hospital after 14 and 10 days respectively. Serum amylase values indicated mild to moderate pancreatic injury without any evidence of other organ damage (Table 2).

Table 2
Values of amylase in the blood of the two surviving cases.

Days after accident	Day 1	Day 2	Day 4	Day 6	Day 10	Day 14
Case 4	142	429	186	335	244	Discharge
Case 5	98	87	–	–	Discharge	

IU/L: 58–166 IU/L in normal level.

4. Animal experiment

We carried out an animal experiment to toxicologically clarify the effect of lime sulfide poisoning on the pancreas by analyzing tissue samples of whole pancreas. Equal number of male and female in each Group, 10-week-old C57BL/6J mice (Sankyo Labo Service, Tokyo, Japan) with an average weight of 18–20 g were used

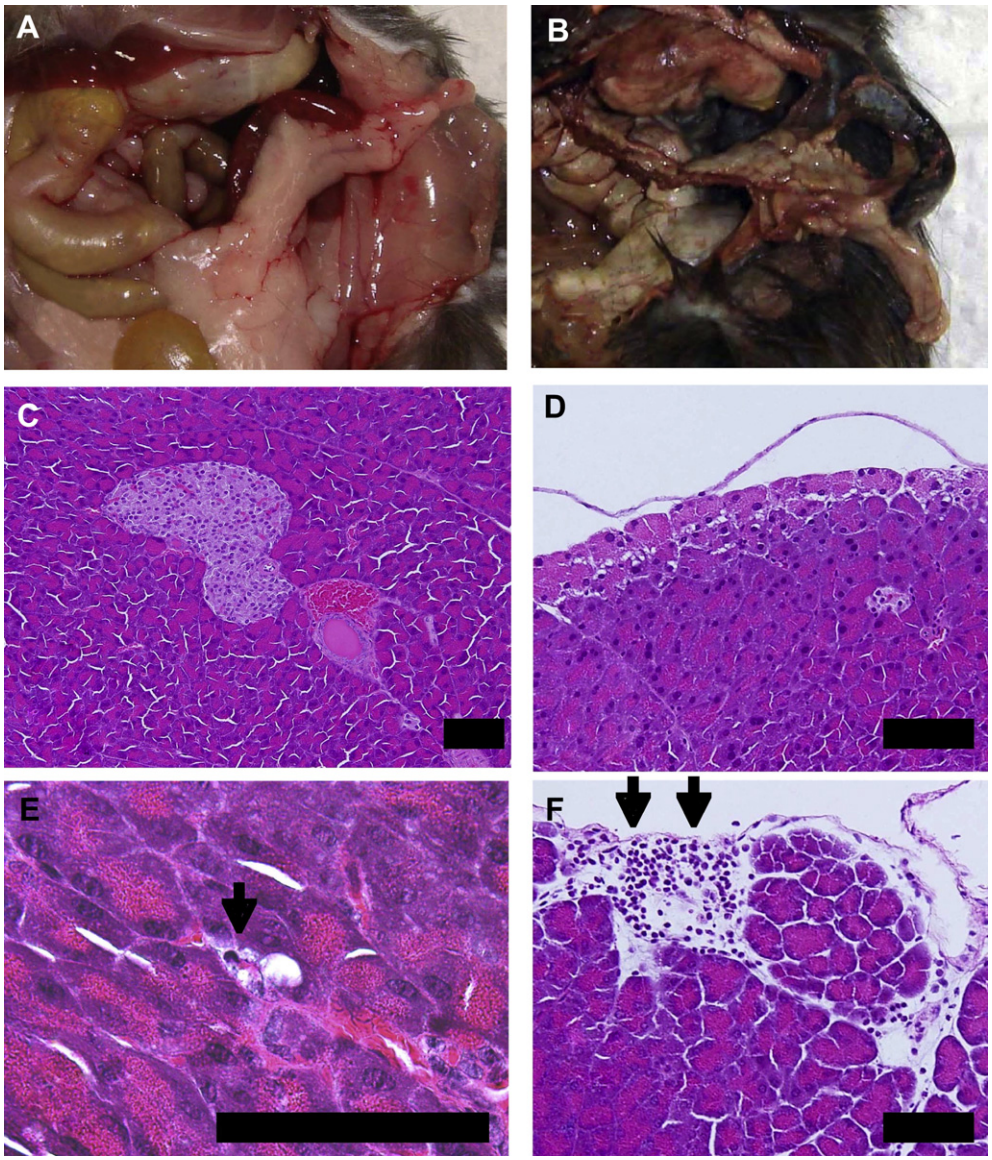

Fig. 2. Animal experiment. A. Normal pancreas of C57BL/6J mouse. B. Gross appearance of pancreas in Group III. C.Microscopic picture of normal pancreas in Group 1. D. Microscopic picture of pancreas in Group IV. E. Microscopic picture of pancreas in Group II. Minimal necrosis in perivascular area with neutrophil infiltration. F. Microscopic picture of pancreas in Group III. Acute inflammation causing severe necrosis. Arrows indicate infiltrated neutrophils. H&E. Bar = 100 μm.

in the experiment. The agent used was generic calcium sulfide (Otoh Co., batch no. 7696, Yamagata, Japan). It is employed as an insecticide, fungicide, and acericide exclusively in gardening and commercially available throughout Japan. In conventional use it is diluted 7–200-fold with water. It was exactly the same agent as consumed in Case 1, in undiluted form. In the animal experiment this agent containing 25% of calcium polysulfides (CaS_5, MW = 183) was administered by intravenous injection (i.v.) or intraperitoneal injection (i.p.) to the mice. Concentrations of lime sulfide were set for Group I–IVs each containing 4 mice, according to previous experiments.[12–14] Group I: 0.13 mL and 0.3 mL volume of physical saline for i.v. and i.p. respectively as a control. Group II: 182 μg/kg mouse weight CaS_5 diluted with saline to 0.13 mL volume was administered by i.v. Group III: 727 μg/kg mouse weight CaS_5 was done by i.p. of low dose. Group IV: 1.45 mg/kg mouse weight CaS_5 was done by i.p. of high dose, based on our human Case 1 who had orally ingested 1.45 mg/kg body weight. The mice were killed 12 h after injection and then the entire pancreas was excised and weighed. Pancreases were formalin-fixed, embedded in paraffin and then sliced and stained with hematoxylin and eosin (H&E) for histological evaluation. The number of infiltrated neutrophils was counted under a light-microscope system (BX51 and DP20, Olympus, Tokyo, Japan) and the percentage of area of necrosis was calculated into an average with software (PhotoshopCS2 9.0.2, Adobe system, CA, USA) after pathologically observing the area of necrosis. The experiment was approved by the Committee on Animal Experimentation of Kanazawa University.

5. Results of animal experiment

Administration of high dose CaS_5 by i.p. caused sudden death of all mice in Group IV, that demonstrated microscopic features of zonal necrosis without any inflammation in the superficial area of the pancreas (Fig. 2D). Surviving mice in Group I–III showed slight to severe swelling in gross appearance. (Fig. 2B). A small number of neutrophils accumulated around necrotic cells in the perivascular area of the pancreas in Group II, while significantly large numbers of infiltrating neutrophils were seen in the superficial area of those in Group III (Fig. 2F; Table 3).

6. Discussion

Inhalation of hydrogen sulfide gas as a cause of death is well known. The cause of death in many such cases has been considered to be acute suppression of the central nervous system and respiration through hydrogen sulfide intoxication generated by the chemical reaction occurring between lime sulfide and acid gastric juice.[2] However, because the volume of gastric juice is limited, the intragastric generation of hydrogen sulfide gas is also limited, and in fact, in cases in which lime sulfides are ingested in large quantities, excessive alkaline passes through the gastric wall and permeates the surface of the pancreas. Thus in cases in which lethal inhalation has been avoided when lime sulfides pass through the posterior wall of the stomach and directly reach the anterior surface of the pancreas, it is mandatory to consider the influence exerted on the pancreas by lime sulfides not only in local blood but also blood in the general circulation.[15,16] Furthermore, in the present non-fatal cases blood and urine lime sulfide concentrations were measured, and at the same time amylase values were followed in hospital, thereby allowing us to verify that pancreatic damage was also present even in patients with only mild sulfide poisoning in the absence of injury to any other organs.[17]

In some cases of acute pancreatitis, which shows a variety of abnormal clinical laboratory data, it is difficult to distinguish cause and effect because of the overlapping final features. Following intake of lime sulfide and inhalation of hydrogen sulfide gas, it is clear that the underlying mechanism of pancreatitis is autodigestion induced by abnormally activated pancreatic enzymes. This appears to be triggered by the influx into the bloodstream of hydrogen polysulfide or directly permeated lime sulfide to the abdominal cavity.

The present experiment elucidated that a survival time of at least several hours after lime sulfide reaches the pancreas is required to demonstrate histological features of acute inflammation such as neutrophil infiltration. This finding is significant because in cases of lime sulfide poisoning in the emergency medicine setting this window of opportunity may suggest scientifically based approaches to suppress later damage to the pancreas.

Last, one additional cause of death that should be considered is the corrosive effect of infiltrating alkali on a large segment of the digestive tract wall, possibly leading to acute injury and necrosis of the wall.[18,19]

Table 3
Results of animal experiment.

Group ($n = 4$)	Course to death	Wet weight of pancreas	Area of necrosis (%)	Count of neutrophils (count/HPF)
I	12 hr, sacrificed	167.4 mg	<0.01	<0.01
II	12 hr, sacrificed	192.7 mg	4.3	1.03
III	12 hr, sacrificed	225.8 mg	17.9	35.6
IV	<30 min, dead	172.1 mg	11.5	0.02
Human Case 1	Several hr (estimated)	106 g	>80	12.09

HDF, high power field.

Conflict of interest
None declared.

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Ethical approval
None declared.

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